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Relative Importance of Ingested Sediment Versus Pore Water as Uptake Routes for PAHs to the Deposit-Feeding Oligochaete *Ilyodrilus templetoni*

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Abstract. The relative role of sediment pore water and ingested sediment particles to the total uptake of sediment-associated hydrophobic organic contaminants was examined by estimation from a water-only exposure experiment and from a bioenergetic-based toxicokinetic model utilizing experimentally measured sediment ingestion rates, assimilation efficiencies, and elimination rates. Phenanthrene (PHE) and benzo[*a*]pyrene (B*a*P) uptake in the bulk deposit-feeding oligochaete, *Ilyodrilus templetoni,* was measured. Assimilation efficiencies (ASE) were measured using a pulse-chase technique, based on a single-gut-passage time. Sediment-associated phenanthrene exhibited a lower ASE (50%) compared to B*a*P (80%), possibly due to a general relationship between assimilation and compound $\log K_{ow}$. Estimated uptake of phenanthrene from pore water alone was essentially equal to the observed total uptake from both ingested sediment and sediment pore water. Estimated contribution of sediment-bound phenanthrene accounted for less than 20% of the total uptake. For benzo[*a*]pyrene, estimated uptake from sediment ingestion accounted for essentially all of the total uptake and estimated absorption from pore water accounted for $<5\%$ of the total uptake. This research provides direct experimental evidence for a predicted increase in the importance of sediment ingestion relative to the pore water route of exposure as the hydrophobicity of organic contaminants increases.

Deposit-feeding invertebrates can take up organic contaminants from surrounding water, either sediment pore water or overlying water, by absorption directly across the body wall or across respiratory surfaces. In addition, contaminants may accumulate from ingested sediment particles by desorption followed by absorption across the gut wall in the presence of digestive fluids (Weston *et al.* 2000). Knowledge of the uptake route of hydrophobic contaminants by deposit-feeding organisms is very helpful in predicting the steady-state body burden, improving sediment toxicity tests, and formulating more precise bioaccumulation models. However, determining the primary uptake route of the hydrophobic contaminants in a sediment matrix is challenging due to the complex physical, chemical, and biological processes involved and their possible interactions within the sediment.

Several methods have been used to quantify the uptake route of organic contaminants. These include comparing tissue concentrations of compounds resulting from exposure in the presence and absence of sediment (Fowler *et al.* 1978; Ma *et al.* 1998; Harkey *et al.* 1994), comparing the uptake of feeding and nonfeeding animals in sediment (Leppänen and Kukkonen 1998), and model predictions using either a bioenergetic-based model, a mass-balance model, or a diffusion-reaction model (Weston *et al.* 2000; Forbes *et al.* 1998; Landrum and Robbins 1990). However, each method has limitations and no studies have been conducted using more than one method to determine the primary uptake route of different compounds in the same species. Based on studies using several species and divergent methods, the dominant uptake route for sediment-associated contaminants with $\log K_{ow}>5$ appears to be from ingested sediment particles (Belfroid *et al.* 1996; Loonen *et al.* 1997; Meador *et al.* 1995). Because different species may respond differently to the same compound, tests on a single species should be done to confirm this predicted relationship.

The polycyclic aromatic hydrocarbons phenanthrene (PHE) and benzo[*a*]pyrene (B*a*P) were investigated. Previous research (Landrum and Robbins 1990; Loonen *et al.* 1997) suggested that PHE and B*a*P are taken up by benthic organisms through different routes (PHE from sediment pore water and B*a*P by ingestion of sediment particles); differences have been related to their differing hydrophobicity. The model used in the current study was a bioenergetic-based toxicokinetic model (Norstrom *et al.* 1976) that assumes uptake from each route is independent and additive. It has been used to determine the uptake of contaminants from different routes, especially from sediment ingestion with experimentally measured model parameters (Weston *et al.* 2000; Boese *et al.* 1990). The purposes of this study were to examine the primary uptake route of sediment-associated PHE and B*a*P in *I. templetoni* and to

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compare the results from water-only exposure and uptake via sediment ingestion.

Materials and Methods

General Method

In this study, the primary uptake route of two PAH with different chemical properties was studied in the same species, the freshwater bulk deposit-feeding oligochaete, *Ilyodrilus templetoni* (Southern). Total uptake into tissue was measured by monitoring uptake during exposure to contaminated sediment. Total uptake into worms burrowed in sediment potentially includes both pore-water and sediment-ingestion pathways. Absorption of each compound across the body wall from pore water alone was estimated by monitoring uptake in water-only exposures. Uptake from sediment ingestion was estimated by measuring sediment ingestion rates, contaminant assimilation efficiencies, and contaminant elimination rates employed in conjunction with a bioenergetic-based toxicokinetic model.

Total Sediment Uptake of PHE and BaP

The total uptake of PHE and B*a*P while burrowed in sediment was measured by exposing *I. templetoni* to contaminated sediments and measuring tissue concentrations at different exposure periods. The source of sediment, from Bayou Manchac, Louisiana, and the test organism, *Ilyodrilus templetoni,* used in this study were the same as those in previous studies (Lu *et al.* 2003, 2004). The sediment, after sieving, was spiked with the test chemicals using a procedure employed by Thoma (1994). Preweighed tracers were dissolved in hexane, transferred to an inoculation vessel (4-L glass bottle), and evaporated under a stream of nitrogen with rotation of the inoculation vessel. As hexane evaporated, weighed wet sediment was placed into the inoculation vessel. The vessel was tumbled for approximately 3 weeks at an axial rotation rate of 5–10 rpm on a roller mill. The inoculated sediment was desorbed upon completion of tumbling using an isopropanol and electrolyte solution (0.01 *M* NaCl, 0.01 *M* $CaCl_2 \cdot 2H_2O$) at a ratio of 1:1 (v/v) to obtain sediments with different concentrations and distinct fractions associated with the desorption-resistant compartment.

The test sediments used in this study had a PHE concentration of 50.7 μg PHE/g dry sediment and ^{14}C activity of 81.9 dpm/mg dry sediment, and a B*a*P concentration of 25.9 μg B*a*P/g dry sediment and a ^{3}H activity of 414.3 dpm/mg dry sediment, respectively. Preliminary toxicity experiments showed that *I. templetoni* tolerates PHE concentrations as high as 330 μg PHE/g dry sediment without significant lethality for at least 10 days (Lu, unpublished). Additional research suggests that reductions in feeding rate occur when exposed to PHE, however, EC_{50} values were found to be greater than 100 μg PHE/g dry sediment (Gust and Fleeger, unpublished). No mortality or avoidance of sediment was observed when *I. templetoni* was exposed to sediment with 25.9 μg B*a*P/g dry sediment.

Measurements of the uptake of PHE and B*a*P were conducted in 50-ml glass centrifuge tubes. Fifteen *I. templetoni* of similar size and with empty guts (worms were allowed to clear their guts in artificial pond water, 0.5 m*M* NaCl, 0.2 m*M* $NaHCO_3$, 0.05m*M* KCl, and 0.4 m*M* C_aCl_2, for 6 h) were added to each tube with approximately 50 g wet sediment. *I. templetoni* tissue concentrations of PHE and B*a*P were measured in batches at different exposure periods. Three replicates were used at each exposure interval, except for short-term exposures of PHE (less than 2 days). In this instance, a small amount of sediment was used, the experiment was conducted in 25-ml jars, and only two replicates were available due to the more frequent sampling intervals. At each sampling time, *I. templetoni* tissue concentration and radioactivity were analyzed by high-performance liquid chromatography (HPLC) and liquid scintillation counting (LSC) using the procedures described below.

Water-Only Exposure Experiment

This experiment was designed to estimate the uptake of PHE and B*a*P via absorption from sediment pore water. After purging gut contents in artificial pond water for 6 h, *I. templetoni* of a similar size were exposed to spiked water. For the PHE experiment, two concentrations, 200 and 500 μg PHE/L, were used to investigate the effect of concentration on uptake. No mortality was observed at either concentration. The dosed solutions were made by diluting the spiked solution of unlabeled PHE (Sigma, St Louis, MO, USA). In initial experiments with B*a*P, the water concentration was 2 μg B*a*P/L (half the water solubility of B*a*P) made by mixing 100 μl ^{3}HB*a*P (American Radiolabeled Chemicals Incorporation, St Louis, MO, USA) with 250 ml water. Toxicity of B*a*P to *I. templetoni* at this concentration was observed. Therefore, the mixture was diluted twice, resulting in a water concentration of 1.84 E5 dpm/ml (~0.43 μg B*a*P/L), and toxicity was not detected. Experiments were conducted in 25-ml glass vials, nine worms were exposed in 10 ml of artificial pond water in each vial, and two replicates were set up at each exposure period. Tissue and water were sampled and analyzed after 0, 0.5, 1, 2, 6, 12, 24, 72, 96, and 145 h exposures for phenanthrene and 0.67, 4, 10, 24, 72, 122, 242 h exposures for B*a*P. Mass balance in the vials at each sampling time was calculated. Uptake from water was quantified by the bioconcentration factor (BCF; tissue concentration divided by water concentration) derived by solving the coupled mass balance equations of the worms and water (Ma *et al.* 1998). Loss from water in the phenanthrene experiment was included in the model, and only one elimination rate was used because previous results showed weak biotransformation of PHE and B*a*P (Lu 2003) (see Table 1 for measured biotransformation fraction of PHE and B*a*P). The mass balances for worms and water are as follows.

Concentration changes in water:

$$V_w \frac{dC_w}{dt} = -k_w C_w M_t + k_e C_t M_t - k_{\text{loss}} V_w C_w \quad (1)$$

Concentration changes in worm:

$$\frac{dC_t}{dt} = k_w C_w - k_e C_t \quad (2)$$

where C_t is the contaminant tissue concentration (μg PHE/g dry tissue or dpm B*a*P/mg dry tissue); C_w, the water concentration (μg PHE/L or dpm B*a*P/ml); V_w, the total volume of water (ml); t, time (h); M_t, dry weight of worms (mg); k_w, first-order uptake rate constant from water (L/kg · h^{-1}); k_e, elimination rate constant (h^{-1}); and k_{loss}, loss rate constant from water (h^{-1}).

The rate constants in the model were determined by solving the two differential equations simultaneously and then applying an iterative least squares method minimizing the difference between the observed and the predicted concentration profiles. The calculation and optimization were performed by Matlab programming (Matlab 6.1; Mathworks Inc., Natick, MA, USA).

Table 1. Experimentally measured ingestion rate of *I. templetoni,* elimination rate, biotransformation fraction, and assimilation efficiency of PHE and B*a*P

Compound	Ingestion rate ($mg \cdot mg^{-1} \cdot d^{-1}$)	Elimination rate (h^{-1})	Biotransformation fraction (%)	Assimilation efficiency
PHE	3.9	0.042	17.3 (±5.5)	50%
B*a*P	2.0	0.0066	3.7 (±0.9)	80%

At the apparent steady state, BCF can be derived from Eq. (2):

$$\mathrm{BCF} = \frac{k_w}{k_e} \tag{3}$$

Using this BCF and the pore water concentration of the sediment, we can estimate the uptake of PHE and B*a*P due to absorption from pore water, assuming that uptake from pore water and in water-only exposures is similar.

Uptake via Sediment Ingestion

A bioenergetic-based toxicokinetic model can be used to predict uptake of organic contaminants from different routes (Weston *et al.* 2000; Boese *et al.* 1990). If we omit the uptake from pore water and assume only sediment ingestion, the model simplifies to

$$\frac{dC_t}{dt} = \mathrm{IR} \cdot \mathrm{ASE} \cdot C_s - k_e C_t \tag{4}$$

where C_t and k_e have the same meanings as defined above; IR is the ingestion rate of worms (mg feces mg tissue^{-1} d^{-1}); ASE, the assimilation efficiency of the contaminant; and C_s, the concentration of ingested sediment (μg/g), approximated by the bulk sediment concentration in this study.

Assuming that sediment concentration, ingestion rate, elimination rate, and assimilation efficiency are constant during the exposure, the integrated form of Eq. (4) is

$$C_t = A(1 - \exp(-k_e t)), \quad \text{where } A = (\mathrm{IR} \cdot \mathrm{ASE} \cdot C_s)/k_e \tag{5}$$

We used Eq. (5) and the experimentally measured values of sediment ingestion rate, contaminant assimilation efficiency, and elimination rate to predict the tissue concentration that can be achieved solely from ingested sediment particles. Comparing this concentration with the observed tissue concentration in the sediment exposure, we again estimated what fraction of the total uptake of the contaminant was due to sediment ingestion.

Ingestion rates were quantified as egestion rates in this study because there is no measurable change in the volume or mass of sediment during gut passage for bulk deposit feeders such as *I. templetoni* (Weston *et al.* 2000). Egestion rates were measured by collecting fecal matter from the sediment surface every other day following the methods of Lotufo and Fleeger (1996). Wet feces were freeze-dried and weighed. Egestion rate was calculated as the dry weight of the feces on a daily basis normalized by the total dry weight of the worms in each tube. The ingestion rate used in the model was the average of the egestion rates for each compound at different exposure periods.

Measurement of the elimination rate constant was performed by first exposing *I. templetoni* to sediment contaminated with either PHE or B*a*P for 7 days. After gut clearance (20 h for phenanthrene and 9 h for B*a*P in a water-only solution), worms were exposed to clean sediment to depurate accumulated contaminant. Tissue concentrations at various sampling intervals were measured. Reductions in tissue concentration were fit to a first-order decay model. The elimination rate (k_e) fitted from the model is independent of the length of gut clearance employed as long as full clearance took place. Our data suggest that a minimum of 4 h is sufficient to ensure complete egestion.

Assimilation efficiency was measured using the pulse-chase feeding technique described by Selck *et al.* (1999) and was based on the direct measurement of ingested ^{14}CPHE and ^{3}HB*a*P compared to that remaining in tissues after complete egestion. Twenty-four *I. templetoni* were first exposed to radiolabeled sediment for 40 min, and the worms were then removed, flushed with water, and divided into two groups: an ingestion group and a depuration group. Worms in the ingestion group were analyzed for ^{14}CPHE and ^{3}HB*a*P immediately and worms in the depuration group were allowed to purge their guts in unlabeled sediment for various periods. Worm body burden was measured, and the fraction of mass of the contaminant remaining in tissues after depuration was calculated at each time interval. Complete egestion was determined from the inflection point in the concentration verses time of the depuration curve. Assimilation efficiency was calculated as the fraction of the remaining body burden at the conclusion of the sediment egestion. More details are provided in Lu *et al.* (2004).

Analytical Procedures

Sediment loading of PHE and B*a*P was measured by ultrasonic extraction and analysis on a Hewlett Packard 1100 series high-performance liquid chromatography (HPLC; Hewlett Packard, Palo Alto, CA, USA). ^{14}CPHE and ^{3}HB*a*P activities in sediment and tissue were counted by a Beckman 6000IC liquid scintillation counting (LSC; Beckman Coulter, Fullerton, CA, USA). Further details can be found in Lu *et al.* (2003).

A slightly different procedure was employed in the extraction of tissue PHE due to the small amount of tissue samples compared to sediment samples. The PHE concentration in tissue was analyzed by the following procedure: three living worms were gently blotted dry on filter paper, weighted on an electrobalance, dried, and ground by mixing with 1.5 g sodium sulfate in an 8-ml glass scintillation vial. Two milliliters of acetonitrile was added to each vial, and the mixture was sonicated for 20 min and left overnight for complete extraction.

Approximately 1 ml of the overlying solvent was taken out by glass pipet, filtrated through 0.45-μm filter, and analyzed by HPLC.

Results and Discussion

Total Uptake from Sediment

I. templetoni phenanthrene tissue concentration increased rapidly during the first 12 h of exposure to contaminated sediment, an apparent drop in accumulation was then observed, followed by a second, slower increase to an apparent steady state after about 6 d (Figure 1). The first stage of the dramatic increase in uptake was perhaps a reflection of uptake of the worms upon entering a new environment and was likely due to uptake via absorption from sediment pore water (Harkey *et al.* 1994). Similar results were observed in our other experiments and by other researchers (e.g., Ma *et al.* 1998). The mechanism that causes this decrease in uptake of phenanthrene, however, is unknown. It is not likely due to the decreased bioavailability of phenanthrene over time that has been observed by other researchers (Landrum 1989; Penry and Weston 1998) because tissue concentrations subsequently increased, suggesting that changes may be a biological response. The rapid decrease in tissue concentration might reflect a delay associated with the onset of elimination of phenanthrene due either to bioacclimation or to elimination processes or to mass transfer resistances associated with elimination. The latter process may be associated with uptake by absorption onto the relatively large external surface area of the organism, while elimination may require diffusion through the organism's lipids and release into the smaller internal surface. It also may be due to the short-term decrease in biological activity while acclimating to a new environment.

Unlike PHE, total uptake of B*a*P increased monotonically until achieving a steady state after approximately 1 month of exposure (Lu *et al.* 2004). This is considerably longer than required for achievement of steady-state uptake in PHE and is consistent with previous observations (ASTM 1997).

Uptake via Absorption from Water

Uptake of PHE and B*a*P from pore water alone was estimated through water-only exposure experiments (Figure 2). For PHE, the uptake curve from water was similar to the curve from sediment: a dramatic increase followed by an apparent decrease, then a slow increase. Similar mechanisms as discussed in the preceding section may also contribute to this decrease. Ma *et al.* (1998) attributed it to the nonequilibrium conditions between organisms and pore water. Unlike uptake in the presence of sediment, uptake from water in the water-only exposure never reached steady state due to a rapid evaporative loss of PHE at longer exposures. After 24 h, PHE concentration in tissue and water decreased dramatically, and a mass balance was not obtained. At 96-h exposures, only 60% of the total PHE could be accounted for, and at 145 h, the balance decreased below 50%. Due to evaporative losses, the bioconcentration factor (BCF) was estimated only from data before 24 h.

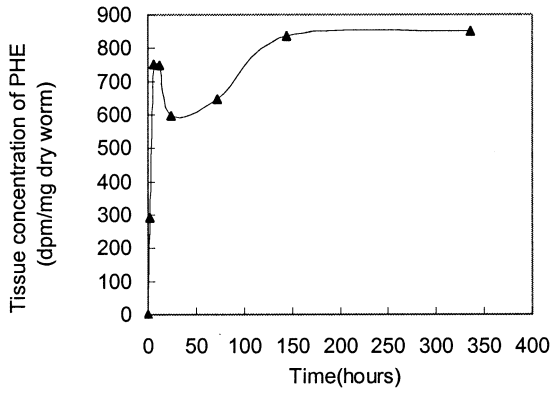

Fig. 1. Total uptake of phenanthrene from sediment at different exposure periods

BCFs for PHE obtained for the high and low water concentrations were 4030 and 4150, respectively, suggesting that uptake of phenanthrene from water was dose independent at the concentration range examined. Using the BCF derived above and the pore water concentration of the sediment, we can estimate the approximate uptake from sediment pore water when steady state is reached (Fowler *et al.* 1978). This uptake was compared with the observed total uptake from sediment (Figure 3). The average BCF was 4090, which is approximately equal, but slightly greater than the BCF calculated from the steady-state body burden and sediment pore water concentration in the total uptake experiment (3080 ± 510). Considering the experimental errors involved in the water-only exposure experiment, uptake of PHE via absorption from water was consistent with the observed total uptake from sediment, indicating that the primary uptake of PHE was from sediment pore water.

Uptake of B*a*P in water-only exposures achieved steady state much more rapidly (after approximately 7 days) than in the presence of sediment (Figure 2). Negligible loss of B*a*P from water was observed during the 10-d exposure, and mass balance was >90% during the entire exposure period. BCF of B*a*P derived from Eq. (3) was 2580 and was <5% of the BCF calculated from the sediment-exposure experiment (80,000). Compared to the total uptake of B*a*P from sediment, uptake of B*a*P from sediment pore water can only account for approximately 3% of the body burden (Figure 3). The results of the water-only experiment on PHE and B*a*P of this study were consistent with the study of Ma *et al.* (1998).

The bioconcentration factor of B*a*P from water-only exposure experiment was lower than the BCF of PHE, which was unexpected from theoretical predictions and general observations that BCF increases with increasing chemical hydrophobicity (Chiou *et al.* 1977; Meader *et al.* 1995). The observed low BCF for B*a*P was likely caused by an apparent high concentration due to the presence of dissolved organic carbon (DOC) in the water. DOC is believed to behave similar to sediment organic carbon, and B*a*P bound by DOC caused the apparent high concentration, which resulted in a smaller than expected BCF. This effect, however, should not be significant for compounds with lower hydrophobicity including PHE. When the BCF was computed with the estimated free PAH, it became more positively correlated to K_{ow} and much closer to

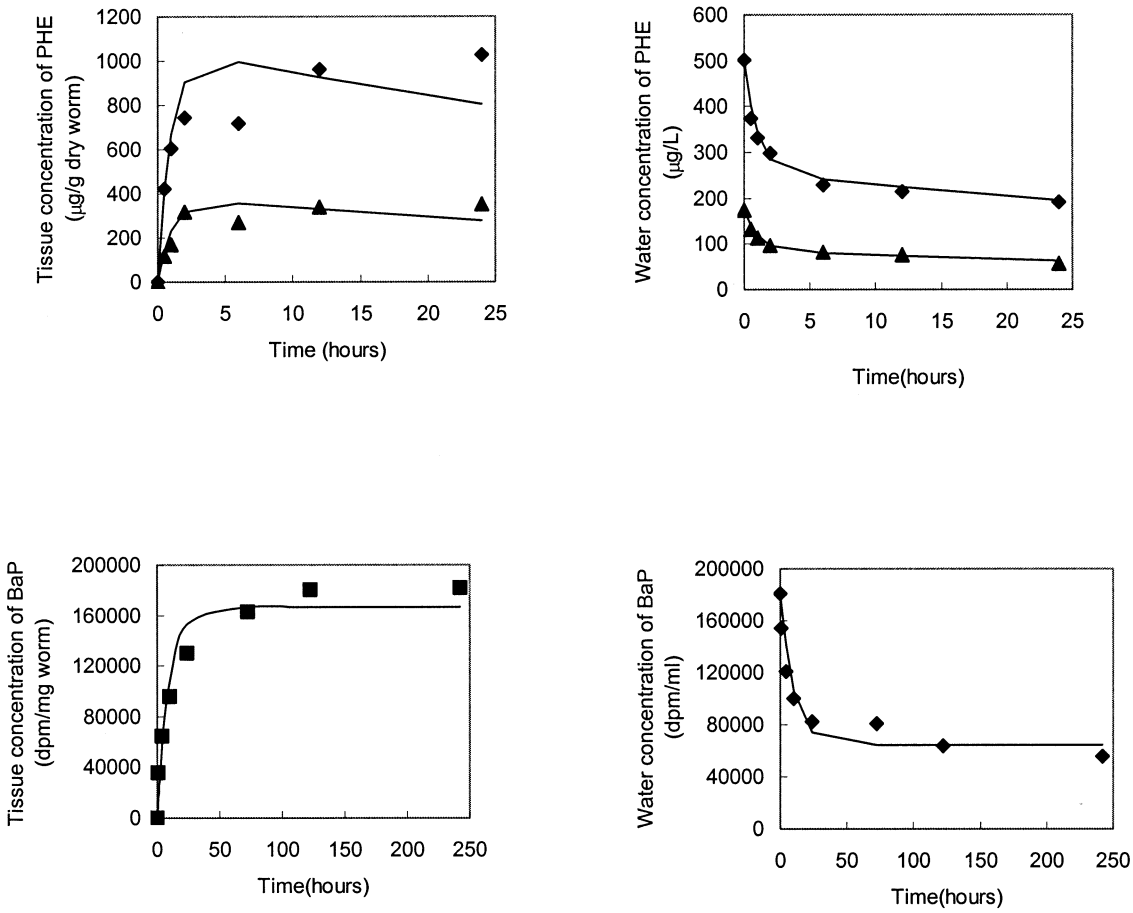

Fig. 2. Changes of phenanthrene and benzo[*a*]pyrene concentration in worms' tissue and water versus time. The scattered data are experimental results and the solid lines are the fit by the mass balance of tissue and water (Eqs. [1] and [2]). The two groups of data on phenanthrene represent two water concentrations (500 and 200 μg/L)

what was expected (Di Toro *et al.* 1991; Meader *et al.* 1995). DOC content was not measured in this study, but measurement of DOC in the water phase of desorption vials strongly supports this hypothesis (Lu 2003).

Uptake via Sediment Ingestion

Like other deposit-feeding oligochaetes, *I. templetoni* ingests large amounts of sediment, processing up to 10 times its own body weight (dry) in phenanthrene-spiked sediment per day (Fleeger, unpublished). In this study, the average ingestion rate of *I. templetoni* in PHE-amended sediment was approximately 4 mg sediment mg dry tissue^{-1} d^{-1}, and it did not change significantly with time after the 4-d exposure. Although no mortality was observed in the B*a*P-spiked sediment, less feces was observed at the surface of the sediment and the average ingestion rate was only approximately 2 mg sediment mg dry tissue^{-1} d^{-1}, potentially indicating some effect of B*a*P in the feeding of the worms (Table 1).

The measured assimilation efficiency of PHE (50%) was lower than B*a*P (80%). The observed higher assimilation efficiency of B*a*P compared to PHE was consistent with the positive trend of assimilation efficiency with logK_{ow} of various hydrocarbons that has been observed by Gossiaux *et al.* (1998). However, there is also some other research showed that assimilation efficiency was negatively correlated with logK_{ow} of hydrophobic compounds (Niimi and Palazzo 1986). This conflict is likely due to the relative importance of the processes associated with contaminant assimilation: uptake involving desorption from sediment particles and partitioning into the lipid of the organisms and loss processes due to egestion and degradation. If assimilation is controlled by desorption of contaminant to the gut fluid, then the highly hydrophobic compound will have lower assimilation efficiency due to the lower desorption rate associated with its higher hydrophobicity. If partitioning to the lipid phase controls the assimilation of the contaminant, however, the more highly hydrophobic compound will exhibit higher assimilation efficiency due to its high partitioning potential. In addition, a higher elimination rate of the less hydrophobic compounds may cause lower assimilation for these compounds, and steric hindrance by the large molecular compounds may restrict their assimilation, although the high hydrophobicity suggests a higher assimilation efficiency. It is, therefore, likely that the relationship between ASE and K_{ow} is curvilinear, as shown by Fisk *et al.* (1998). When

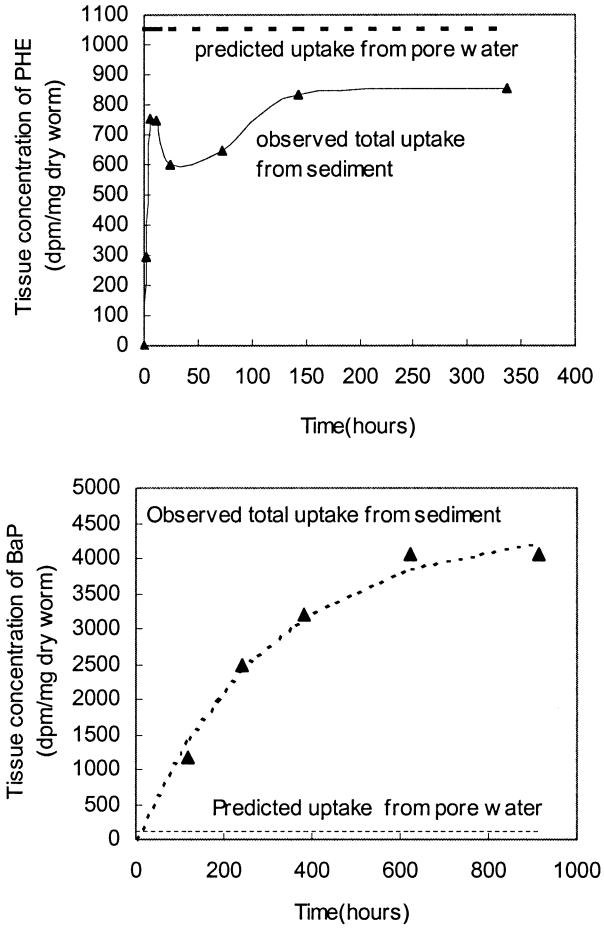

Fig. 3. Comparison of the predicted uptake of phenanthrene and benzo[*a*]pyrene from sediment pore water with the observed total uptake

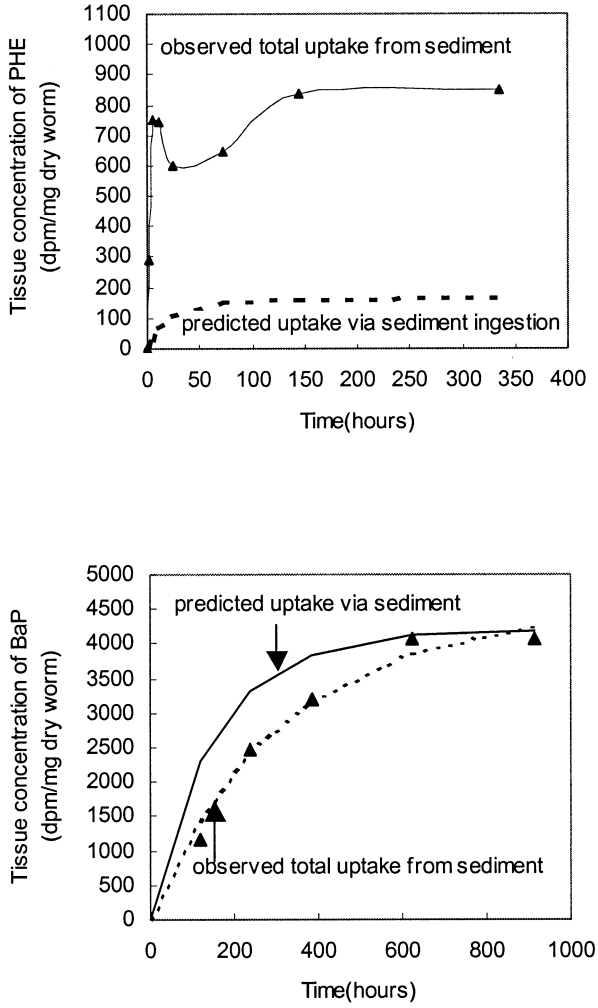

Fig. 4. Comparison of the predicted uptake of phenanthrene and benzo[*a*]pyrene via sediment ingestion versus the observed total uptake

$\log K_{ow} < 7$, e.g., PHE and B*a*P, a positive relation between ASE and K_{ow} is expected from Fisk *et al.* (1998) and was observed in this study.

With the measured ingestion rate, elimination rate, assimilation efficiency, and sediment concentration, we can estimate uptake of PHE and B*a*P that derived solely from ingested sediment particles (Figure 4). Uptake of PHE assuming only sediment ingestion accounted for less than 20% of the total observed uptake at the apparent steady state, and this fraction was even lower during the first stage of rapid uptake of phenanthrene. The measured uptake of B*a*P from sediment ingestion was in agreement with the observed total uptake from sediment. Even at the earliest stages of uptake, uptake from sediment ingestion appeared to dominate total uptake. This finding differs from the work of Weston *et al.* (2000), who found that early uptake of B*a*P for the polychaete *Abarenicola pacifica* was primarily from pore water. *I. templetoni* ingests much smaller particles than *A. pacifica,* which may account for a greater uptake from sediment particles. The predicted uptake of B*a*P via sediment ingestion in *I. templetoni* was greater than the observed total uptake during most of the exposures, which might be due to the high assimilation efficiency of B*a*P and the assumption that assimilation efficiency was a constant during the whole exposure time. Assimilation efficiency likely declines as individuals approach steady state as demonstrated by Weston (1990). Then the predicted uptake of B*a*P via sediment ingestion will be in more agreement with the observed total uptake from sediment.

We used assimilation efficiency (ASE) rather than absorption efficiency (AE) as some authors have done in bioenergetic models. Repeated attempts to measure AE with a dual-label technique using ^{51}Cr- and ^{14}C-labeled PAH with tubificids in our laboratory have been unsuccessful using a single-gut-passage technique (Fleeger, unpublished) because essentially no chromium appears in the ingested sediment group after feeding for the length of time of a single gut passage even though labeled PAH is detected. The result is an AE $>100\%$. Lydy and Landrum (1993) showed that another selective deposit feeder also did not take up ^{51}Cr and attributed it to either selective ingestion of particles or entry of labeled PAH via pore water. ASE should be similar to AE in the single-gut-passage time experiments we conducted because the loss of PAH from tissues during the 4-h depuration was slow and therefore min-

Table 2. Total organic carbon content (TOC) in sediment and feces

TOC	Clean sediment	PHE[a]			B*a*P
In sediment (%)	1.35 (0.03)	1.22 (0.02)	1.17 (0.01)	1.21 (0.01)	1.20 (0.02)
In feces (%)	1.78 (0.08)	1.45 (0.10)	1.60 (0.19)	2.13 (0.45)	1.31 (0.03)

[a]The first column of phenanthrene data represents the sediment used in this study. Data shown in the other two columns were collected from a previous study (Lu 2003).

imal. If our measure of ASE were inflated by entry from the pore water, this would inflate the contribution from sediment, suggesting that it may be lower than our estimate of 20% for PHE.

We used the bulk sediment concentration of PHE and B*a*P in calculations of the bioenergetic-based toxicokinetic model to represent their concentrations in ingested sediment. This assumption may not be correct, for deposit feeders such as *I. templetoni* tubificid oligochaetes typically ingest a disproportionate fraction of small particles enriched in PAH relative to preingested, bulk sediment (Klump *et al.* 1987; Millward *et al.* 2001). If so, our estimates of uptake from sediment ingestion, especially for PHE, would be underestimated. Fecal particle size distribution measurements suggest that *I. templetoni* is only a moderately or slightly selective feeder. Comparisons of the distributions of particles in bulk sediment and feces demonstrated that *I. templetoni* accumulated a slightly greater mass of particles <63 μm. Most conspicuously, particles <1.5 μm were more common, while particles from 1.5 to 6.0 μm were depleted in the feces (Lu 2003). We cannot be sure if this increase in particles <1.5 μm in feces was due to feeding selectivity or to a breakage of larger particles associated with digestive processes. However, the total organic carbon content of sediment and feces differed by less than a factor of 2 for PHE and by 10% of B*a*P in *I. templetoni* (Table 2). If differences in bulk sediment and fecal organic carbon accurately measure selectively for moderately selective or slightly selective organisms as demonstrated by Kukkonen and Landrum (1995), the maximum uptake of PHE through sediment ingestion would range from 20% with no feeding selectivity to 40% at maximum selectivity. Assuming maximum selectivity, the uptake of PHE by *I. templetoni* would still be dominated by the uptake from sediment pore water. The full extent of selectivity is difficult to evaluate because information on the distribution of PAH, especially in particles <63 μm, is poorly known. The predicted uptake of PHE and B*a*P via sediment ingestion was in agreement with the prediction by Landrum and Robins (1990), who suggested that sediment ingestion accounts for 12% of PHE uptake and almost 100% of B*a*P uptake by *Lumbriculus variegates.*

Comparison of the Two Methods

The uptake routes of sediment-associated PHE and B*a*P were estimated using water-only exposure and sediment ingestion experiments. Results of both techniques proved consistent. The water-only exposure experiment was simple and direct but was limited to short exposure times (many bulk deposit feeders suffer rapid and significant mortality in water in the absence of sediment) and is an unnatural lifestyle in which these worms may alter their behavior. In addition, the precision of this method at long exposure times was poor for phenanthrene due to its instability in water. The method for estimating uptake from sediment ingestion is limited by assumption of constant assimilation efficiency. In addition, selective feeding and variability in ingestion rate may also limit the precision of the method. Uptake of hydrophobic contaminants from sediment is a complex process and is dependent on the characteristics of sediment and the chemistry of the compounds involved and the biology of the organism involved. Employing both water-only and sediment-only exposures to compare to total uptake provided a more convincing indication of uptake route, because the results were consistent.

Conclusions

The results of water-only exposure experiment and the prediction of uptake via sediment ingestion provided consistent results on the route of uptake of sediment-associated PHE and B*a*P in the tubificid oligochaete, *Ilyodrilus templetoni.* Both methods demonstrated that the primary uptake route of PHE was from sediment pore water. The contribution from sediment ingestion accounted for less than 20% of the observed total uptake. Both results showed that more than 95% of the uptake of B*a*P came through sediment ingestion even during the initial stages of uptake. These results suggested that the route for hydrophobic contaminants varies with their chemical properties. Model estimates by Thomann *et al.* (1992) demonstrated that ingested sediment can be the dominant uptake route for hydrophobic compounds with $\log K_{ow} > 5$. The results of this study support that conclusion. The results presented herein, however, are unique in that two different compounds were compared in the same organism via two different independent experimental measurements.

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